QuantWorm software package

Last update on 9/6/2015

User Manual

I. Copyright & Disclaimer

The QuantWorm software package is freeware. Anyone can install the software and modify its source code at home, college, school, or any other public places, but you use the software at your own risk. The software package is distributed 'as is.' No warranty of any kind is expressed or implied. The software is licensed under Academic Free License (AFL) with the following restriction: QuantWorm software is licensed only for non-commercial purposes.

II. Overview of the Software Package

The 'QuantWorm' software package is composed of eight individual software programs written in Java.

- ***WormScanner:*** takes image/video
- ***WormLifespan:*** counts moving worms
- ***WormLocomotion:*** measures worm velocity
- ***WormLength:*** measures worm body length
- ***WormEgg:*** counts eggs
- ***WormCounter:*** counts worms
- ***WormTrapAssay:*** counts worms from video for a worm trap assay
- ***WormGender:*** counts male and hermaphrodite worms

III. Hardware Setup

The system builds upon the *stage* and *camera* components of Worm Tracker 2.0 (which was developed by Eviatar Yemini at the Schafer lab: http://www.mrc-lmb.cam.ac.uk/wormtracker/). The setup that we utilized is as follows:

1. ***Microscope:*** Olympus model SZ61 trinocular stereozoom microscope.
2. ***Motorized stage:*** H105 ProScan from (PRIOR Scientific)
   * Zaber and Ludl stages should also work.
3. ***Digital camera:*** Unibrain Fire-i 501b (IEEE 1394 connection)
   * USB cameras should also work as long as they are properly detected by Windows XP, Vista, or Windows 7.

4. ***Light source:*** fiber optic transmitted light.
5. ***Stage control module:*** ProScan II Controller (PRIOR Scientific)
6. **Compatible plates:**
    - 6 cm and 10 cm petri-dish plates
    - 6-well, 12-well, and 24-well plates

IV. Software Installation

The software package can be run on both Mac and Windows (XP or 7) computers. Since individual software in the package has been developed as a separate program (meaning no dependency between software for its operation), a user does not need to install all of the software but can choose one or two software among them. In case of the WormLocomotion software, a user should install the Java Media Framework API (JMF) for video analysis.

V. WormScanner Software

o Introduction

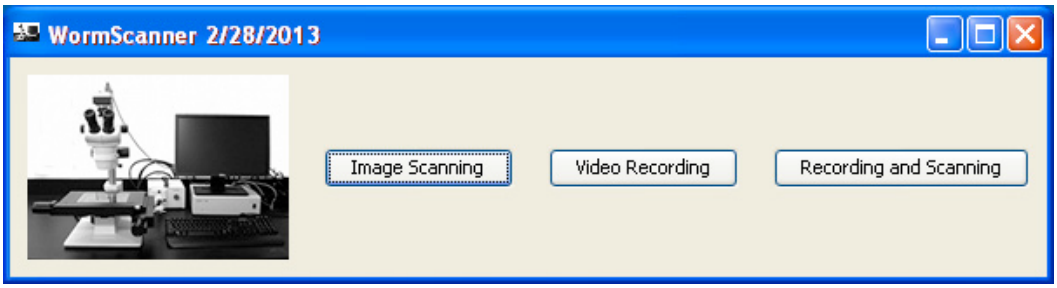
WormScanner is an automated image and video acquisition software that also controls a motorized stage. The software takes multiple piece images (dimension: 640 × 480 pixels), which are used to create a single large assembled plate image. The software also can collect individual well images/videos in a multi-well plate.

o *Compatible plate types*

The software supports 6 cm, 6-well, 12-well, and 24-well plates.

o *User interface and functions*

The WormScanner provides three different methods to take image or videos: ‘Image Scanning,’ ‘Video Recording,’ and ‘Recording and Scanning’. The 'Recoding and Scanning' mode allows the user to take both images and videos of the same well/plate.



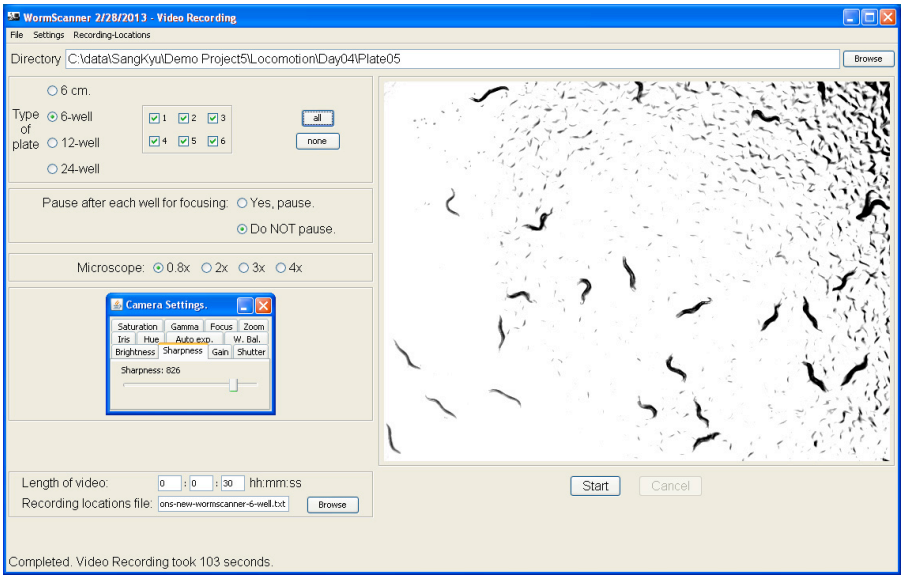
- Image scanning mode

The application window has several useful options such as plate alignment, plate type, number of scanning (once or twice), time interval if case of scanning twice, and camera setting (see below). Before taking images, a user has to (1) set a proper magnification and adjust focus in a microscope; (2) fill all options on the left hand side of the dialog box; (3) align a plate (run the 'Alignment' function in the option menu to define the center of the plate); (4) adjust image brightness and contrast (change light intensity of a fiber optic illuminator and adjust brightness and sharpness levels in the 'Camera Option' menu). Start scanning by clicking on the 'Start' button.

When scanning each well or plate, the software creates a new folder containing all piece images ('piece_*n*.jpeg' where *n* is the sequence number).

- Video recording mode

The user interface of the 'Video Recording' is similar to that of the 'Image Scanning.' The application window offers several useful options such as plate alignment, plate type, video recording time, recording location, and camera setting (see below). The default recording location is the center of the designated plate, however, a user can define new locations in a plate by using the 'Recording Locations' function in the menu. Video recording is initiated when a user clicks on the 'Start' button.



Recording and scanning mode

Combines functions of image scanning and video recording modes.

VI. WormLifespan Software

o ***Introduction***

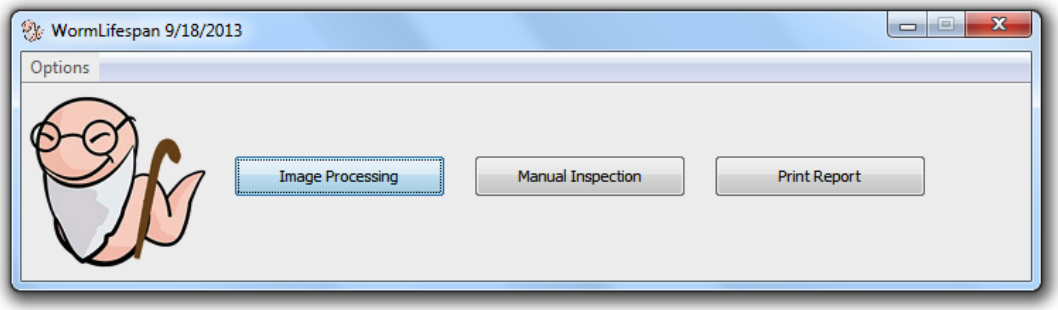
The WormLifespan software counts moving worms by analyzing two consecutive images taken with a certain time interval at the same spot on a plate. Since living worms continuously move or change their body shape, moving worms are detected by comparing two consecutive images.

o ***Taking images***

We recommend taking images at 8X where 1 pixel in image corresponds to approximately 21~24 μm. For the lifespan assay, two image sets should be taken with a certain time interval. We recommend conducting lifespan assays in 6-well plates at a 2-minute time interval.

o ***User graphical interface and functions***

The software has three command buttons as shown below. Use the commands in order from left to right.

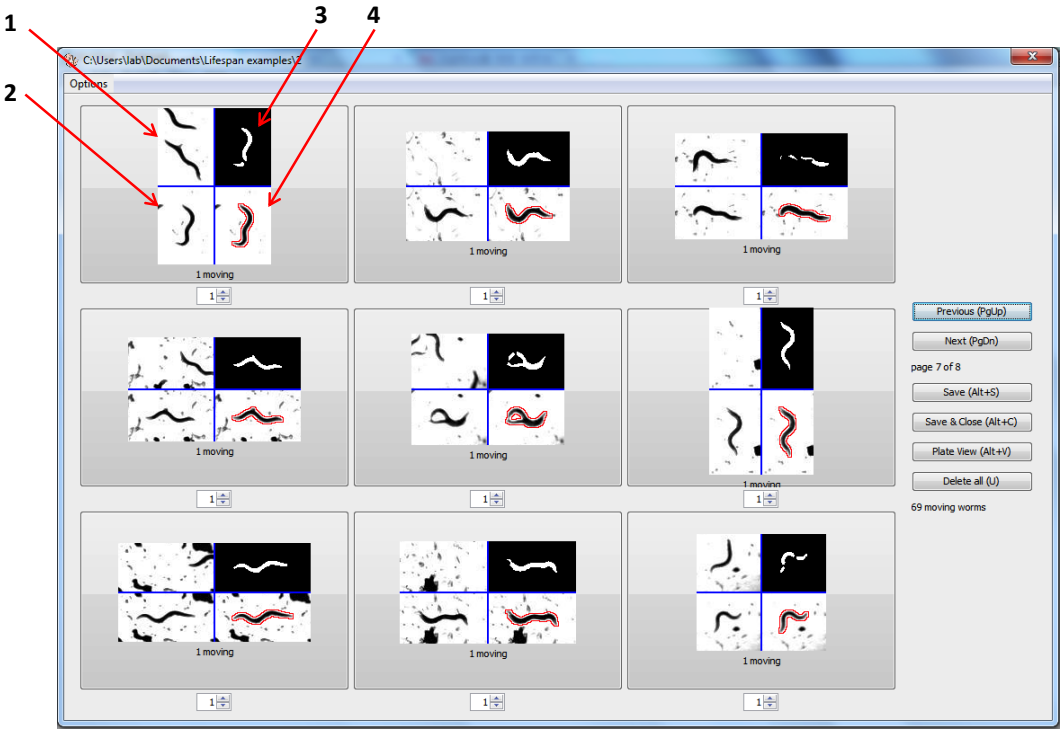


- Image Processing

The purpose of the 'Image Processing' is to conduct batch processing to analyze multiple well or plate images. If a user clicks on the 'Image Processing' button, the software will show a pop-up window where the user chooses a source image folder. The software then automatically navigates to subfolders, processes images and finally writes a result file ('result-lifespan.txt') at every folder where plate images are found.

- Manual Inspection

The software has two built-in manual inspection windows to help a user check results of image analysis and correct errors in fully automated analysis. First, the user can check individual findings in the inspection window (see below).



1: first source image: unmodified source image

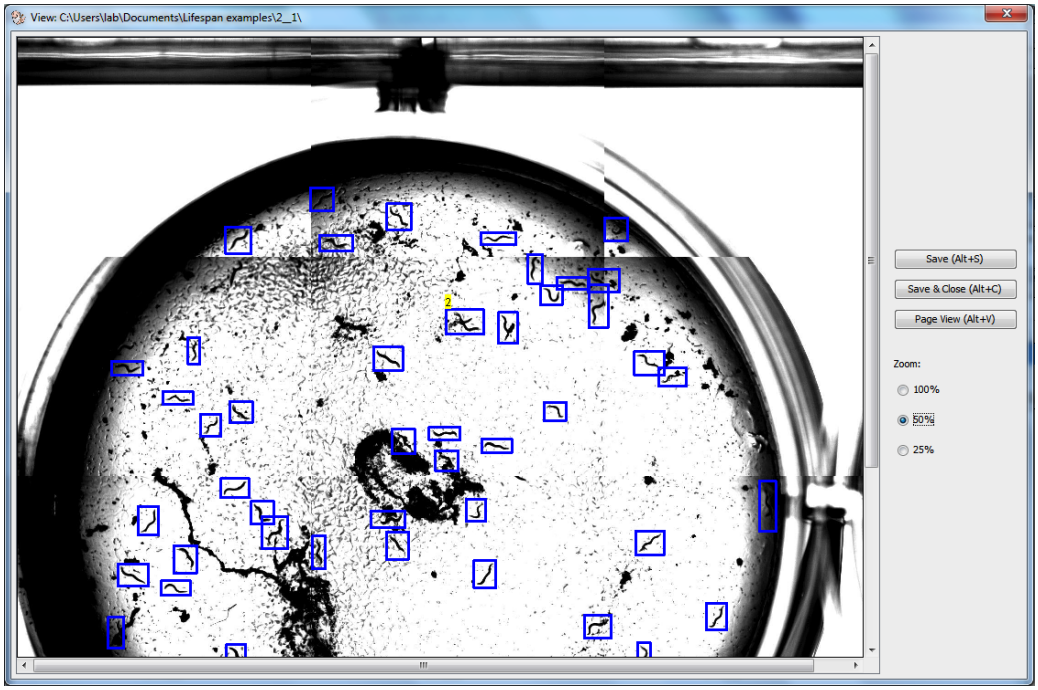
2: second source image: unmodified source image

3: binarized differential image: binarized subtracted image between the first and second source image. White regions show moving parts of a detected object

4: edge detection image: detected object in the second source image shown in red-outline

If any detected object is a false positive, click on the panel image to delete it. If there is more than one worm moving, use the up/down buttons below the panel to adjust the number of worms present or type the number into the box.

Second, the user can add missing worms or delete invalid findings in the plate view inspection window where an entire plate image is animated by switching two time-lapse images.



Once the manual inspection is completed, click on the 'Save' or 'Save & Exit' button to write a result file ('result-lifespan.txt'). In the file, 'nLive' means the number of moving worms in a detected object.

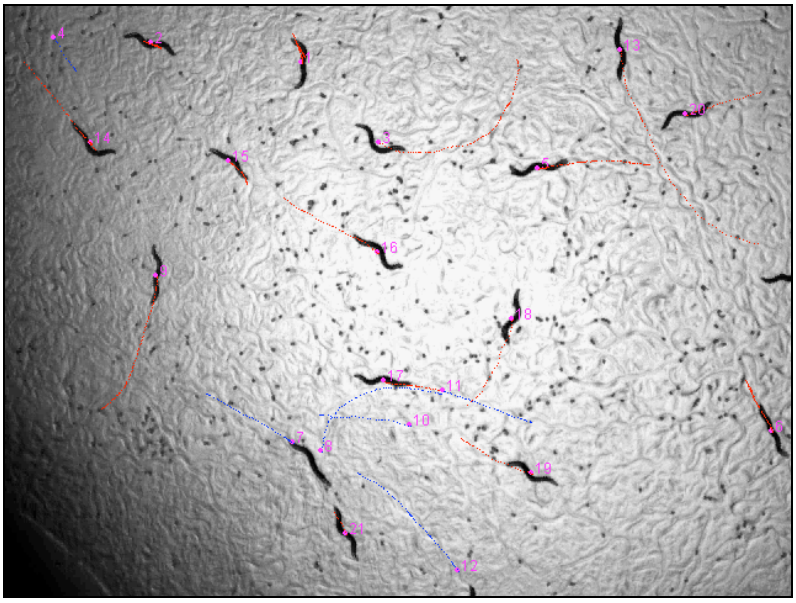
- Print Report

A user can create a summary report by clicking on the 'Print Report.' Once a user specifies a source image folder, the software will navigate to subfolders and then create a tab-delimited result text file ('report-lifespan.txt') containing the number of moving worms in each well or plate.

VII. WormLocomotion Software

o ***Introduction***

The WormLocomotion software conducts fully automated video analysis and computes the velocity of multiple moving worms (see below).

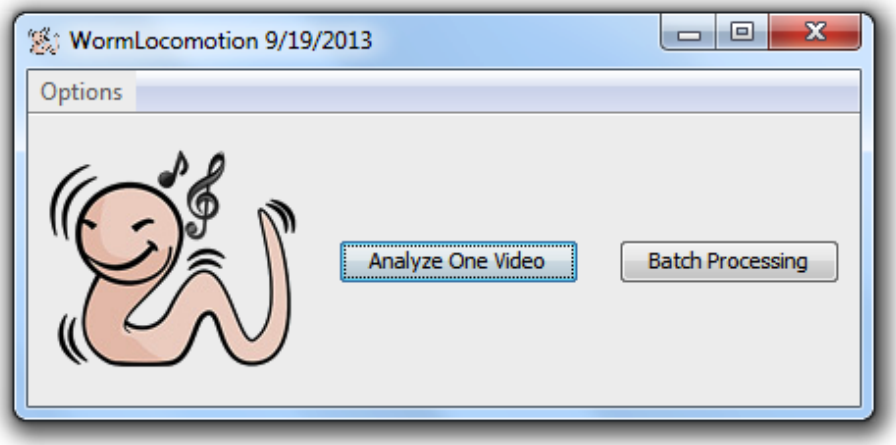


o Taking videos

We recommend taking videos for 30 sec at a lower magnification like 8X where 1 pixel in the video image corresponds to approximately 21-24 μm. A user may want to test a longer recording time if the worms move very slowly.

o User graphical interface and functions

The software has two command buttons as shown below.



- Analyze One Video

If a user wants to analyze a single video file, click on the 'Analyze One Video' button in the window. When the video file is selected, the video pop ups and play. Red tracks are drawn over the video image to indicate the trajectory of moving worms (see below). When video processing is finished, the WormLocomotion software will notify the completion of the operation and show percentile and accumulative percentile histograms. The software also creates two result text files named as 'TrackingResults.txt' and 'TrackingResults_Detail.txt' in the same folder of the source video file. The 'TrackingResults.txt' and 'TrackingResults_Detail.txt' contain the mean velocity of all tracks and the mean velocity of individual tracks, respectively.

- Batch Processing

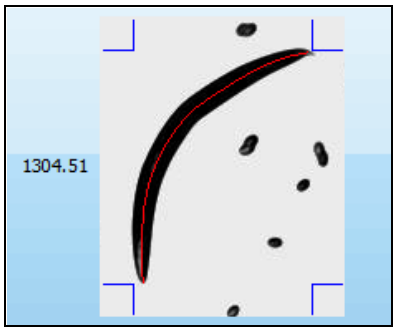
The purpose of the Batch Processing' is to process multiple video files. If a user specifies a source folder, the software automatically goes through all subfolders and conducts video processing at every folder where video files are found. Unlike ‘Analyze One Video,’ individual videos are not displayed during batch processing. When the batch processing is completed, the software creates two tab-delimited result text files ('TrackingResults.txt' and 'TrackingResults_Detail.txt') the same way as in the 'Analyze One Video.'

- Option menu

The software allows a user to choose proper worm detection parameters in the option menu. All detection parameters are stored in the preset file ('Locomotion_Detection_Conditon_Preset.txt') in the folder where the software is installed.

VIII. WormLength Software

The WormLength software measures worm body length from a single image that may contain multiple worms. To estimate worm length, the software extracts a skeleton curve in the middle of each worm.

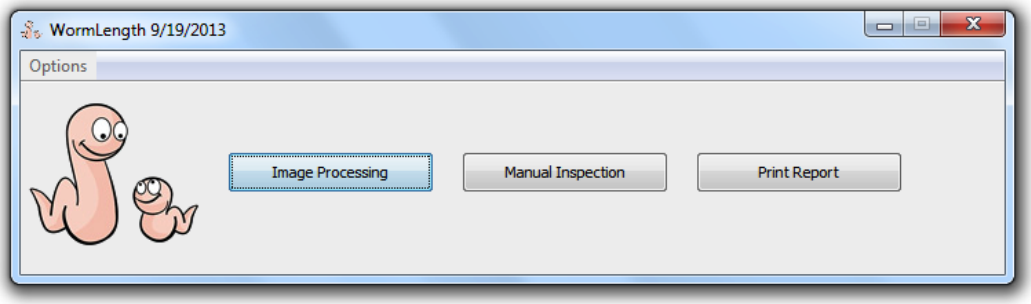


o ***Taking images***

We recommend taking images at 30X magnification (3X objective with 10X eyepiece) where 1 pixel in image corresponds to approximately 6-7 μm. Since worms change their body shape and location very quickly, highly recommend killing worms before taking images.

o ***User graphical interface and functions***

Like the WormLifespan software, there are three command buttons in the application window. Use the software commands from left to right.



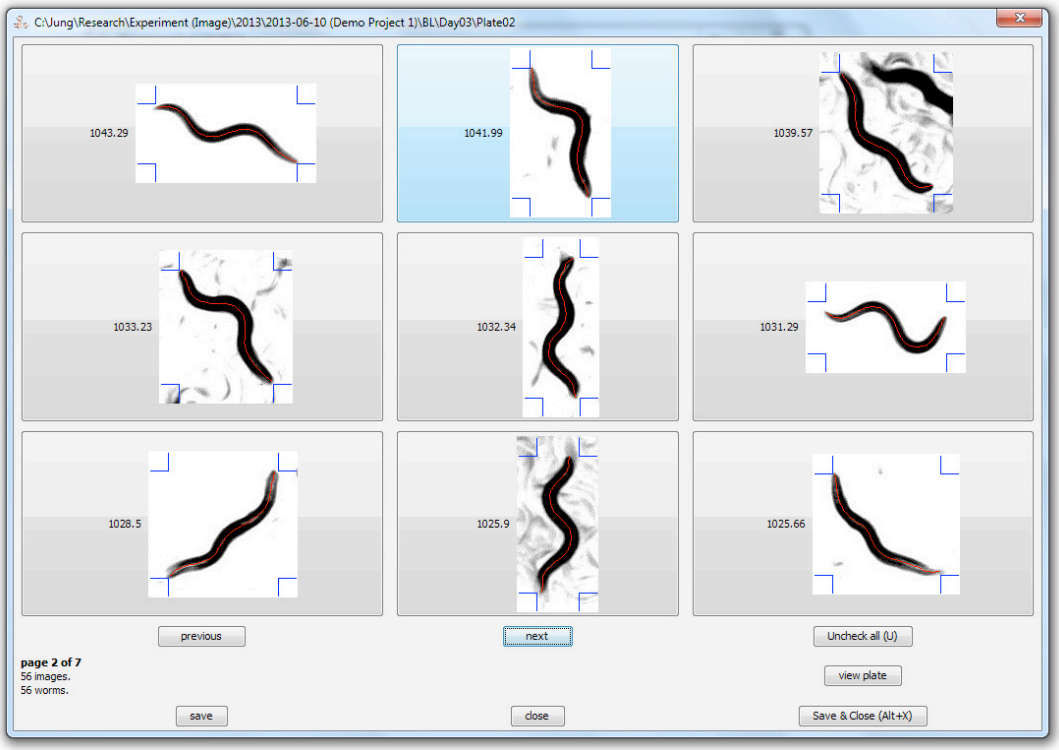
- ***Image Processing***

The purpose of the 'Image Processing' is to conduct batch analysis of multiple well or plate images. If a user clicks on the button, the software will show a pop-up window where the user specifies the source image folder. The software then automatically navigates to subfolders to process images. When image analysis is completed, the software writes a result text file ('result-length.txt') and creates mask images of detected objects, which are found in the subfolder (...\imageClip)

where the images are located.

- ***Manual Inspection***

A user can review the results of image analysis and correct errors made in the fully automated analysis (see below). If any detected object is not a worm or its skeleton curve is wrong, click on the panel image to delete it. Once the manual inspection is done, click on the 'Save' or 'Save & Close' button to write a result file ('result-length.txt').



- Print Report

A user can make a summary report by clicking on the 'Print Report' in the main application window. Once a user specifies a source image folder, the software will navigate to subfolders and then create two result text files ('report-length.txt' and 'report-details-length.txt').

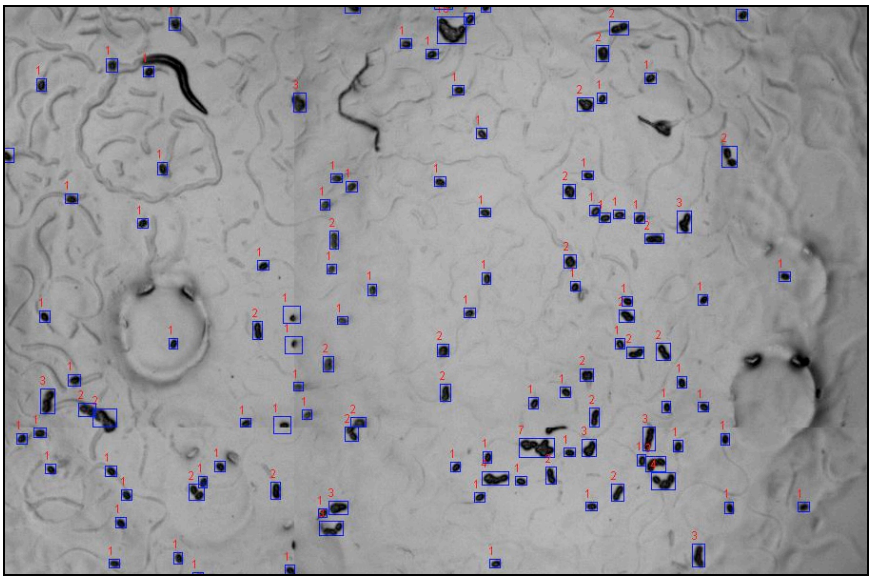
- Option menu

The software allows the user to choose proper worm detection parameters in the option menu. All detection parameters are stored in the preset file ('WormLength_Detection_Conditon_Preset.txt') in the folder where the software is installed.

IX. WormEgg Software

o ***Introduction***

The software was implemented to help a user count eggs from a captured image.

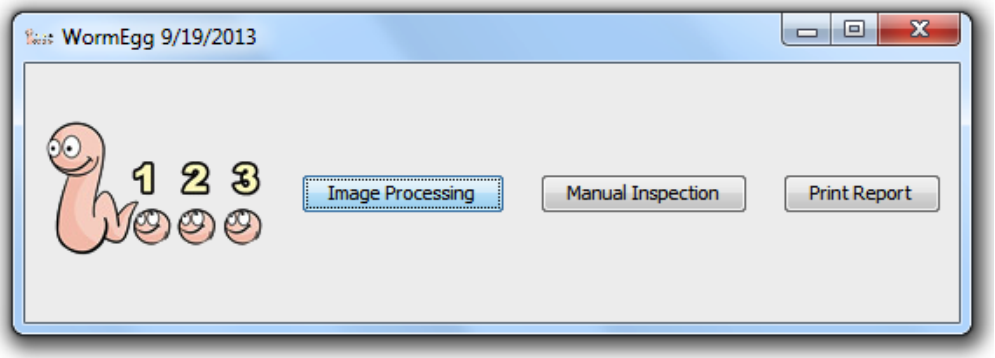


o ***Taking images***

We recommend taking images at 30X magnification (3X objective with 10X eyepiece) where 1 pixel in image corresponds to approximately 6 ~ 7 μm.

o ***User graphical interface and functions***

There are the same three command buttons in the application window. Use the software commands in order from left to right.



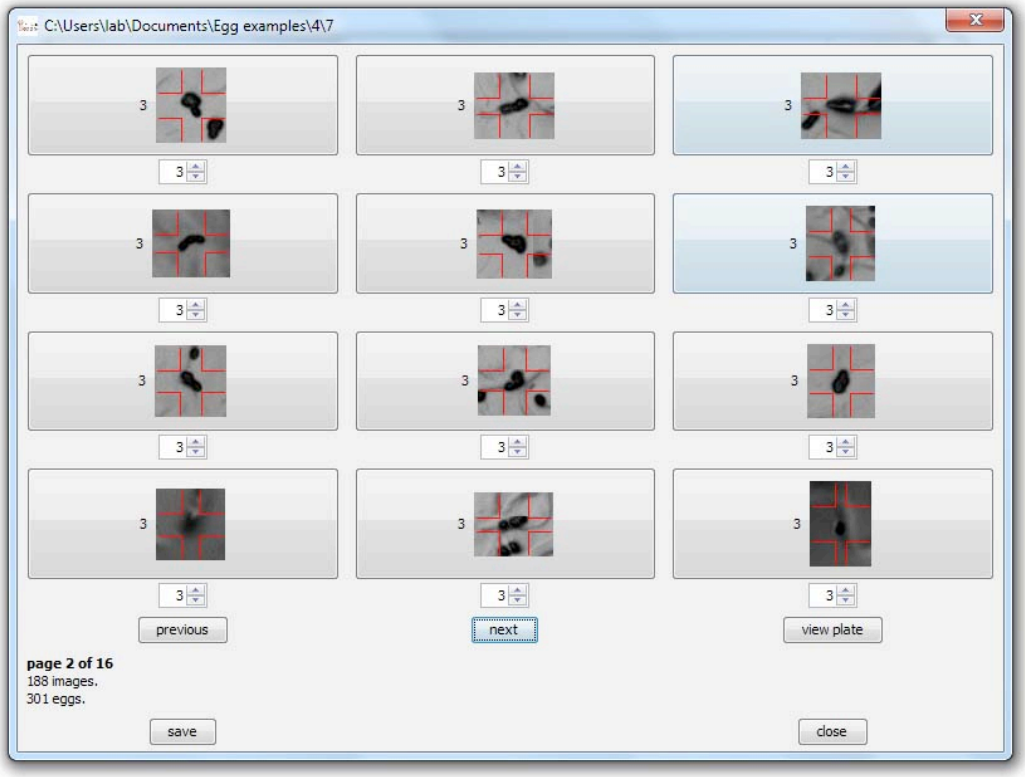
- Image Processing

The purpose of the 'Image Processing' is to conduct batch analysis of multiple well or plate images. If a user clicks on the button, the software will show a pop-

up window where the user specifies source image folder. The software then automatically navigates to subfolders and processes images. When image analysis is completed, the software writes a result text file ('result-egg.txt') at every folder that contains source images.

- Manual Inspection

The user can review the result of image analysis and correct errors in fully automated analysis (see below). If any detected object is not an egg, click on the panel image to delete it. When the manual inspection is done, click on the 'Save' button to write a result file ('result-egg.txt').



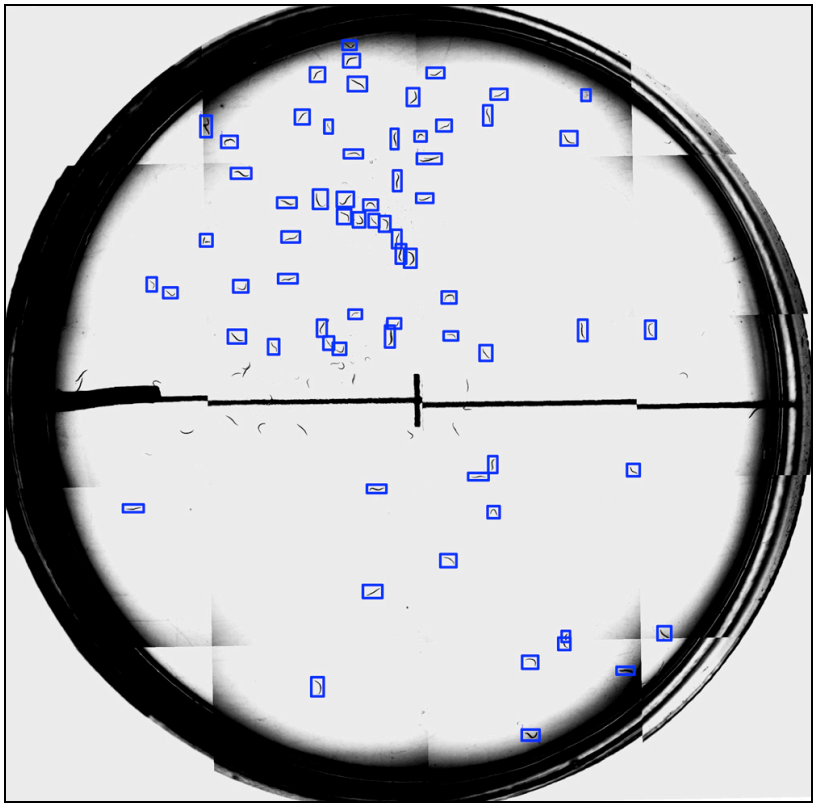
- Print Report

A user can make a summary report by clicking on the 'Print Report' in the main application window. Once a user specifies a source image folder, the software will navigate to subfolders and then create a result text files ('report-egg.txt').

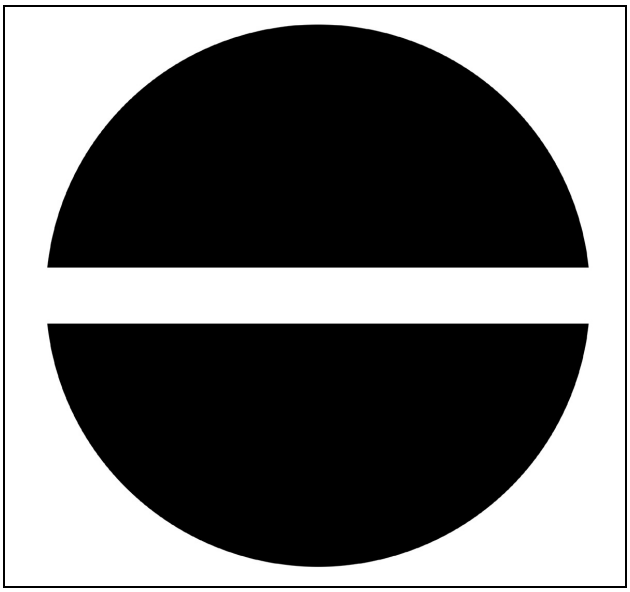
X. WormCounter Software

o ***Introduction***

The software is used to count worms especially for a chemotaxis assay.



As a default, the following mask image ('mask.jpg') is used for counting worms in two different semi circle regions marked as black.

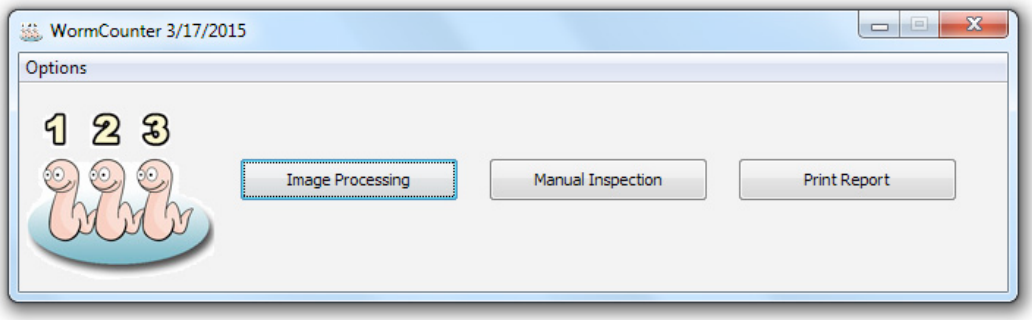


o ***Taking images***

We recommend taking images at 8X where 1 pixel in image corresponds to approximately 21~24 μm.

o ***User graphical interface and functions***

There are three command buttons in the application window. Use the software commands in order from left to right.

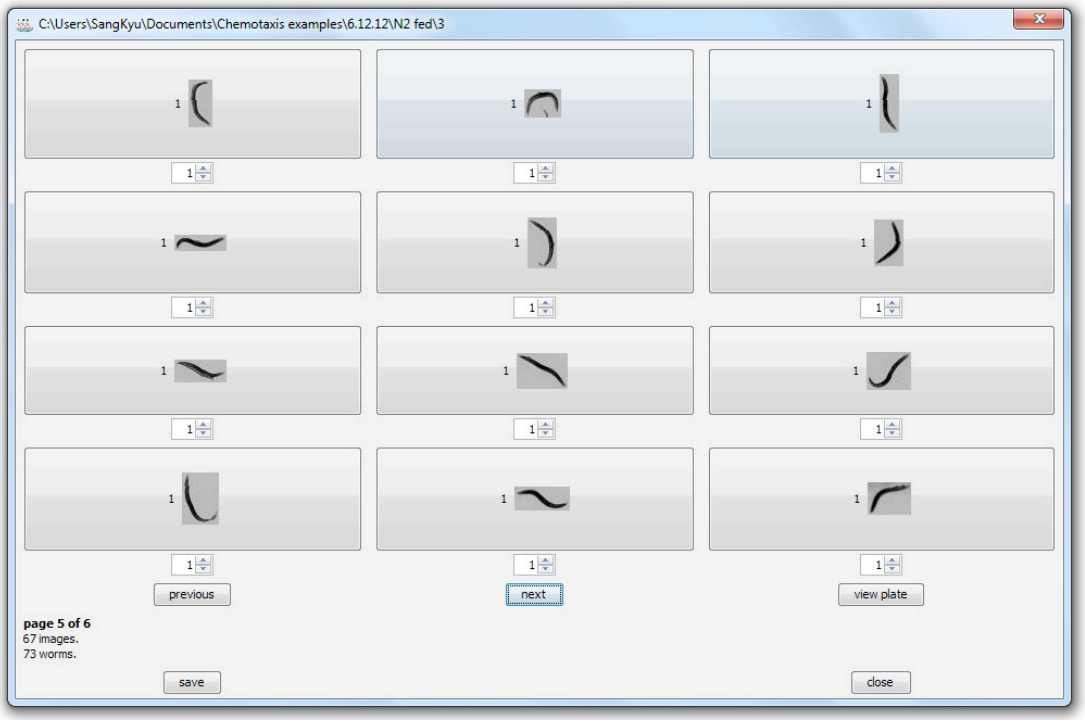


- Image Processing

The purpose of the 'Image Processing' is to conduct batch analysis of multiple well or plate images. If a user clicks on the button, the software will show a pop-up window where the user specifies source image folder. The software then automatically navigates to subfolders and processes images. When image analysis is completed, the software writes a result text file ('result.txt') at every folder that contains source images.

- Manual Inspection

The user can review the result of image analysis and correct errors in fully automated analysis (see below). If any detected object is not an egg, click on the panel image to delete it. When the manual inspection is done, click on the 'Save' button to write a result file ('result.txt').



- Print Report

A user can make a summary report by clicking on the 'Print Report' in the main application window. Once a user specifies a source image folder, the software will navigate to subfolders and then create a result text file ('avoidance.txt').

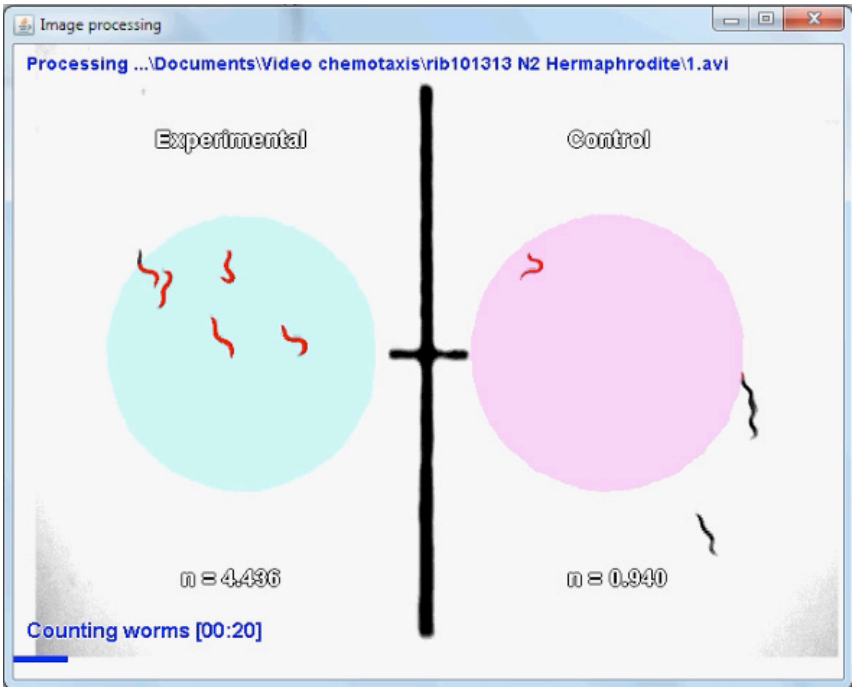
o ***Choosing another mask image***

A user can choose another mask image for image processing. Click on the 'Specify mask' in the 'Options' menu of the software.

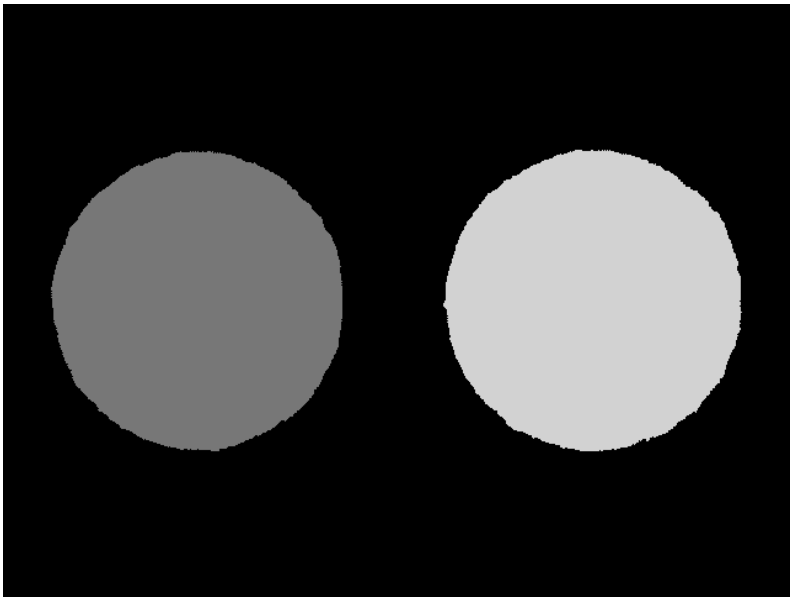
XI. WormTrapAssay Software

o ***Introduction***

The software is fully automated video analysis software for counting worms in two regions.



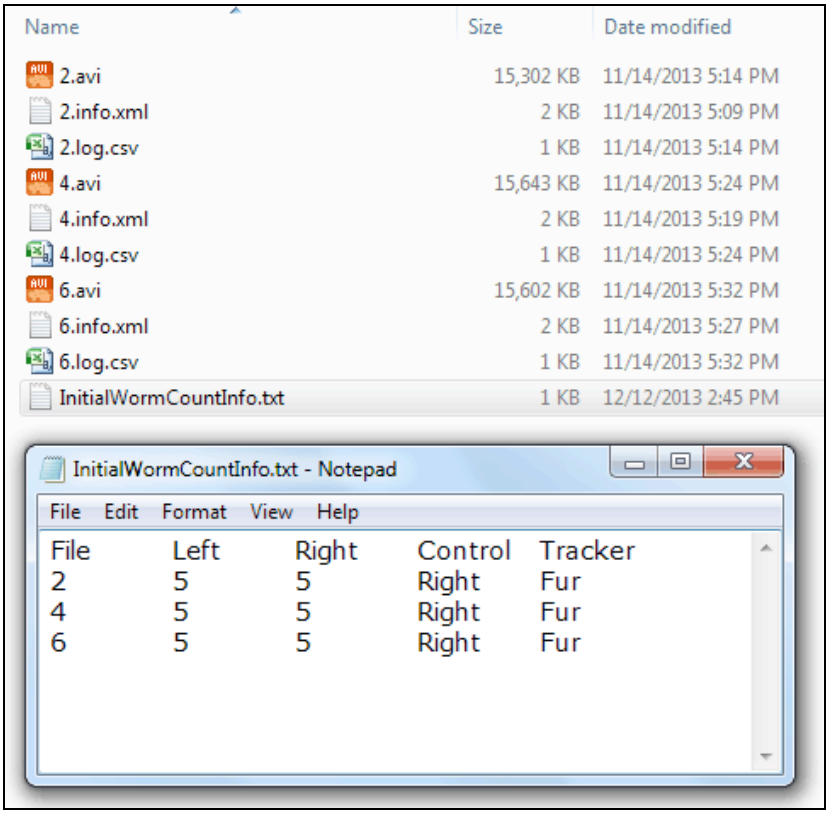
As a default, the following mask image ('mask of fur.gif', 'mask of ish.gif', or 'mask of rib.gif') is used for counting worms in two different semi circular regions.



o ***Taking images***

We recommend taking images at 8X where 1 pixel in image corresponds to approximately 21~24 μm.

Once videos are taken, a user should create a 'InitialWormCountInfo.txt' file in every folder containing video files (see below).


Name
Size
Date modified
2.avi 15,302 KB 11/14/2013 5:14 PM
2.info.xml 2 KB 11/14/2013 5:09 PM
2.log.csv 1 KB 11/14/2013 5:14 PM
4.avi 15,643 KB 11/14/2013 5:24 PM
4.info.xml 2 KB 11/14/2013 5:19 PM
4.log.csv 1 KB 11/14/2013 5:24 PM
6.avi 15,602 KB 11/14/2013 5:32 PM
6.info.xml 2 KB 11/14/2013 5:27 PM
6.log.csv 1 KB 11/14/2013 5:32 PM
InitialWormCountInfo.txt 1 KB 12/12/2013 2:45 PM
InitialWormCountInfo.txt - Notepad
File Edit Format View Help
File Left Right Control Tracker
2 5 5 Right Fur
4 5 5 Right Fur
6 5 5 Right Fur

File: video file name

Left: initial worm count in the left circle region

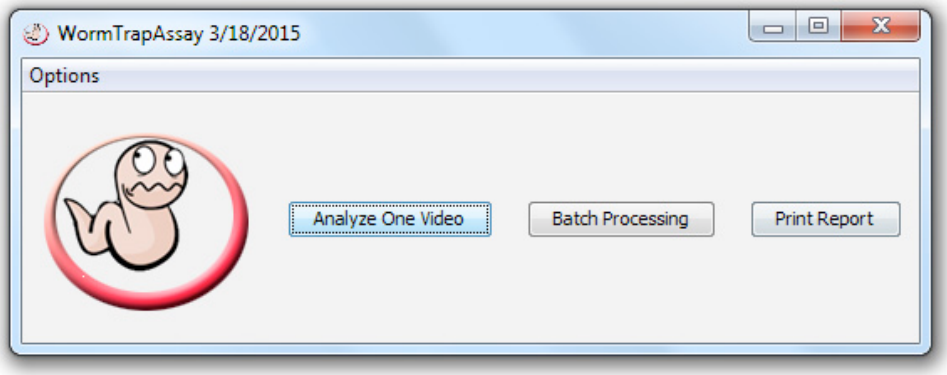
Right: initial worm count in the right circle region

Control: specify which side is the control (Right or Left)

Tracker: specify tracker name. Tracker name is used to select mask image.

o ***User graphical interface and functions***

There are three command buttons in the application window.



- Analyze One Video

Use this command button to analyze a single video file. If a user clicks on the button, the software will show a pop-up window where the user specifies a source video file. When image analysis is completed, the software writes a result text file (*video filename*.WormTrapAssay_Result.txt).

- Batch Processing

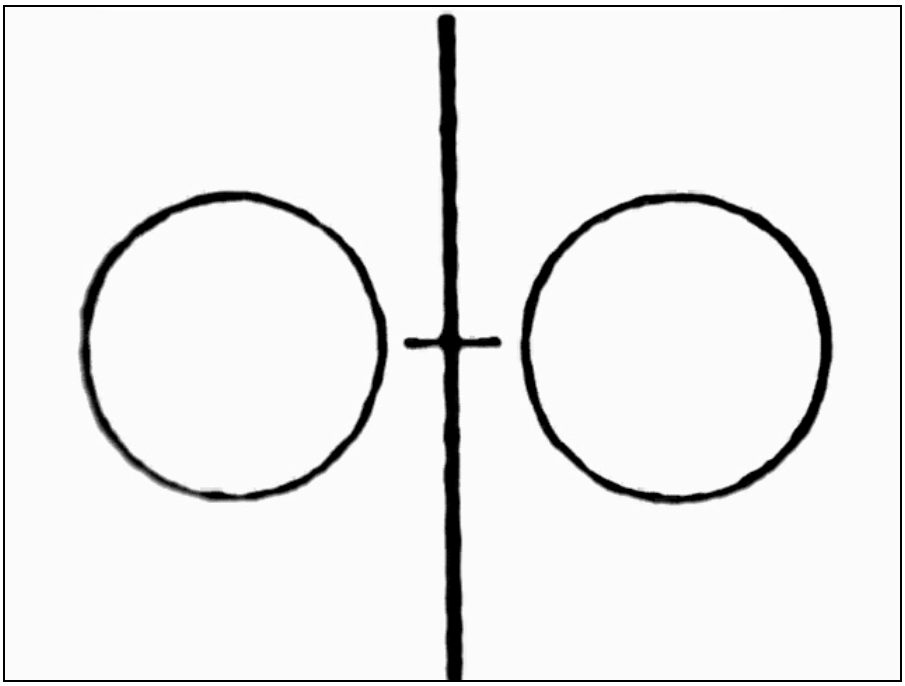
The purpose of the 'Batch Processing' is to conduct batch analysis of multiple well or plate videos. If a user clicks on the button, the software will show a pop-up window where the user specifies a source image folder. The software then automatically navigates to subfolders and processes every video. When the batch processing is completed, the software writes result text file for every video.

- Print Report

A user can make a summary report by clicking on the 'Print Report' in the main application window. Once a user specifies a source image folder, the software will navigate to subfolders and then create a result text file ('Report-WormTrapAssay.txt').

o ***Creating a new mask image***

A user can create a new mask image automatically using the software. First, take a template video without worms (see below).

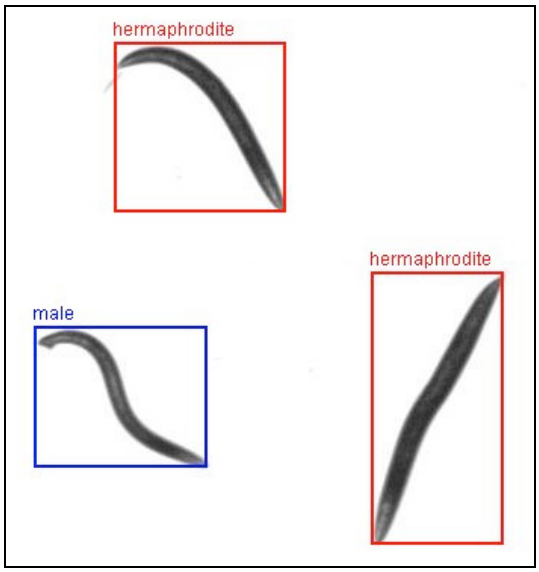


Second, click on the 'Create a new mask image from video' in the 'Options' menu of the software. WormTrapAssay will create a new mask image file ('mask of xxxx.gif') in the folder where the software is installed.

XII. WormGender Software

o ***Introduction***

The software is used to count male and hermaphrodite worms from a captured image.

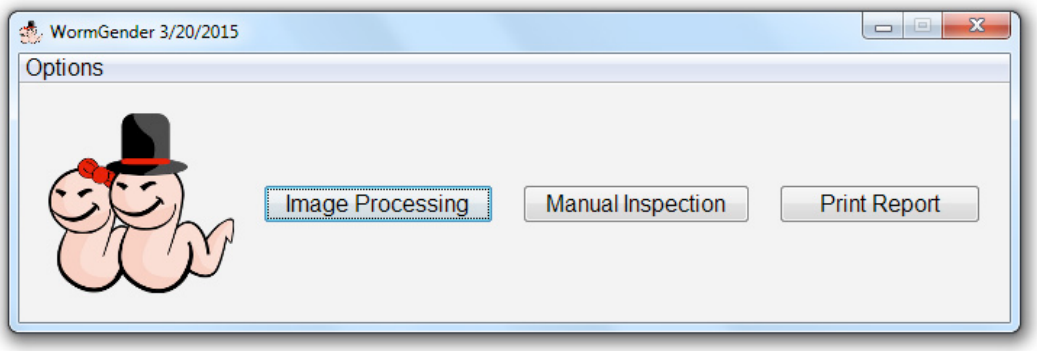


o ***Taking images***

We recommend taking images at 30X magnification (3X objective with 10X eyepiece) where 1 pixel in image corresponds to approximately 6 ~ 7 μm.

o ***User graphical interface and functions***

There are three command buttons in the application window. Use the software commands in order from left to right.



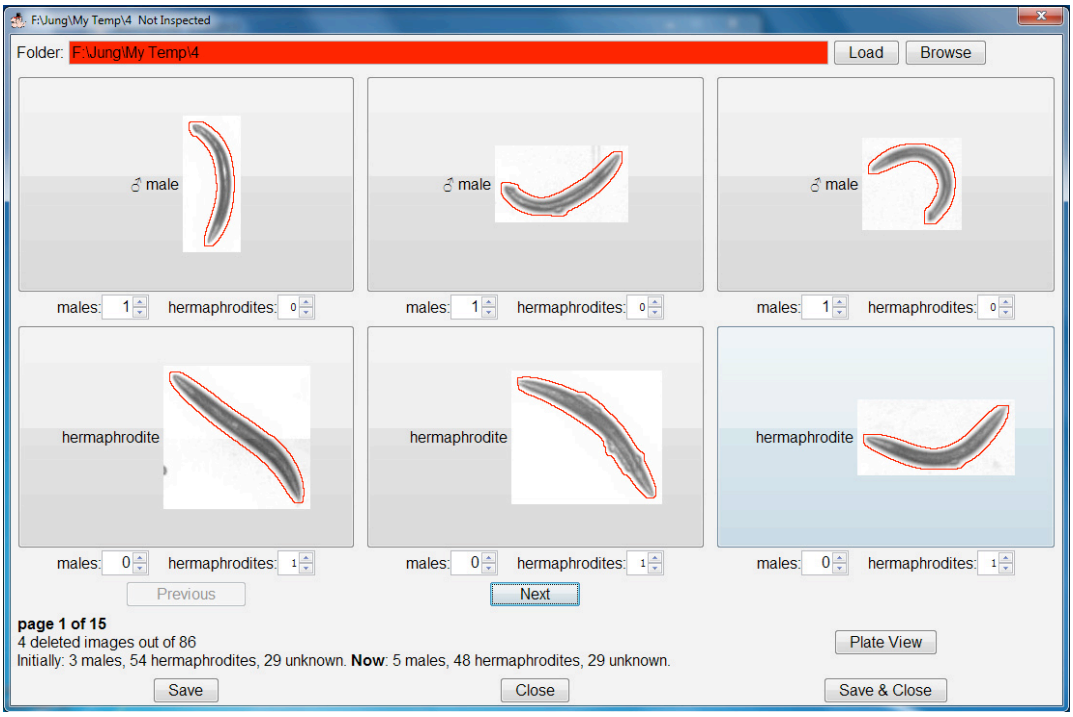
- Image Processing

The purpose of the 'Image Processing' is to conduct batch analysis of multiple

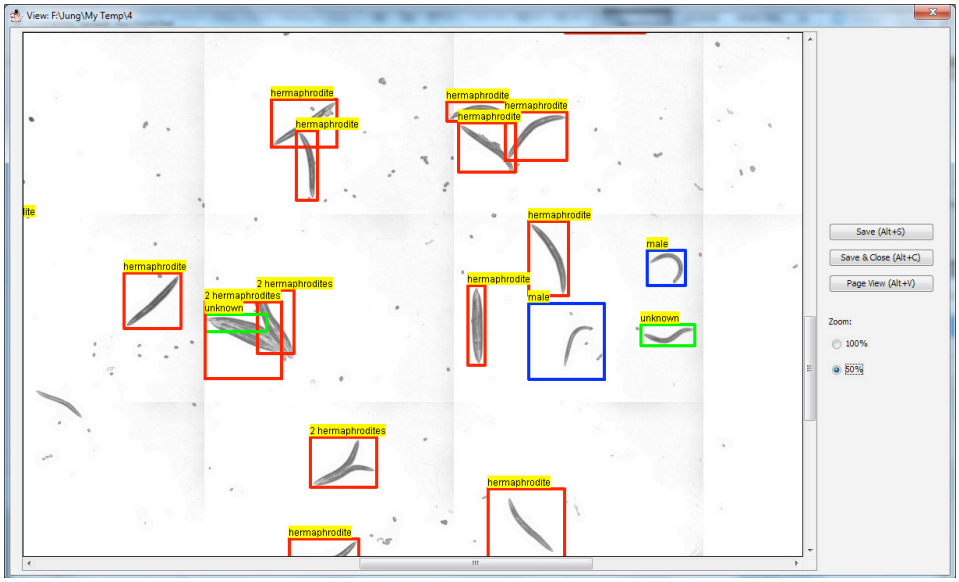
well or plate images. If a user clicks on the button, the software will show a pop-up window where the user specifies source image folder. The software then automatically navigates to subfolders and processes images. When image analysis is completed, the software writes a result text file ('result-gender.txt') at every folder that contains source images.

- Manual Inspection

The user can review the result of image analysis and correct errors in fully automated analysis (see below). If any detected object is not an adult worm, click on the panel image to delete it. When the manual inspection is done, click on the 'Save' button to write a result file.



A user can also inspect image analysis results in the plate view window (see below). Click on the 'Plate View' button in the manual inspection window.

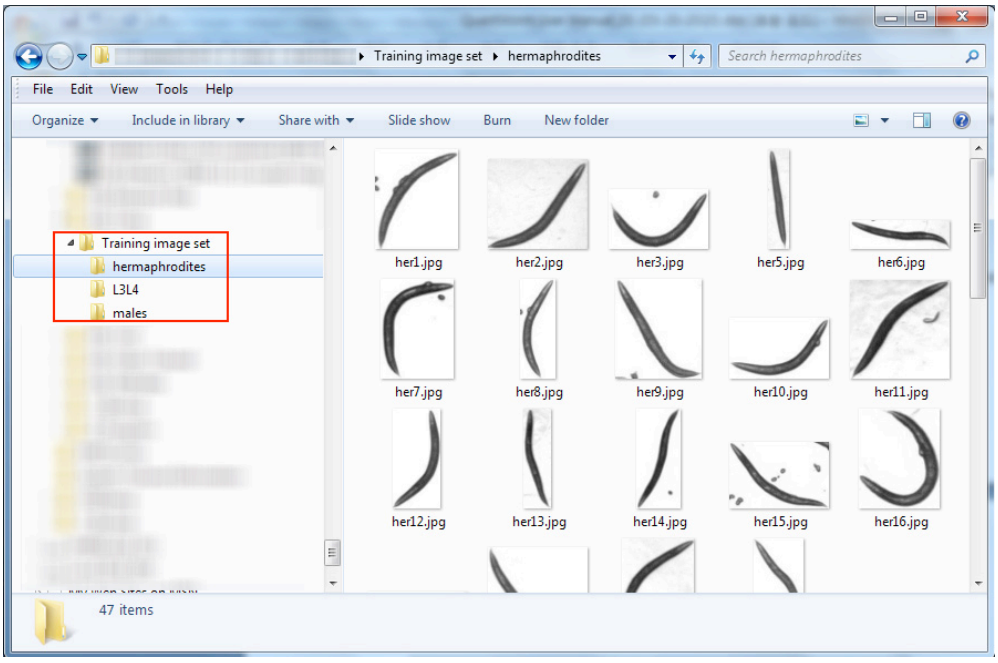


- Print Report

A user can make a summary report by clicking on the 'Print Report' in the main application window. Once a user specifies a source image folder, the software will navigate to subfolders and then create a result text files ('report-gender.txt').

o ***Creating a new training set***

A user can create a new training set file ('TrainingSet.txt') using the software. First, create individual worm image files under specific folders. Note that the following training image set is already included in the software (zip) or sample images (zip).



Use the same folder names: hermaphrodites, L3L4, and males. Worm image files should be saved as 'jpg'.

Click on the 'Analyze and Create New Training Set' in the 'Options' menu of the software and then choose the 'Training image set' folder.

WormGender software will create a new training set file ('TrainingSet.txt') in the 'Training image set' folder. To use the new training set, the file should be located in the folder where WormGender is installed before running the software.